

FIG. 1

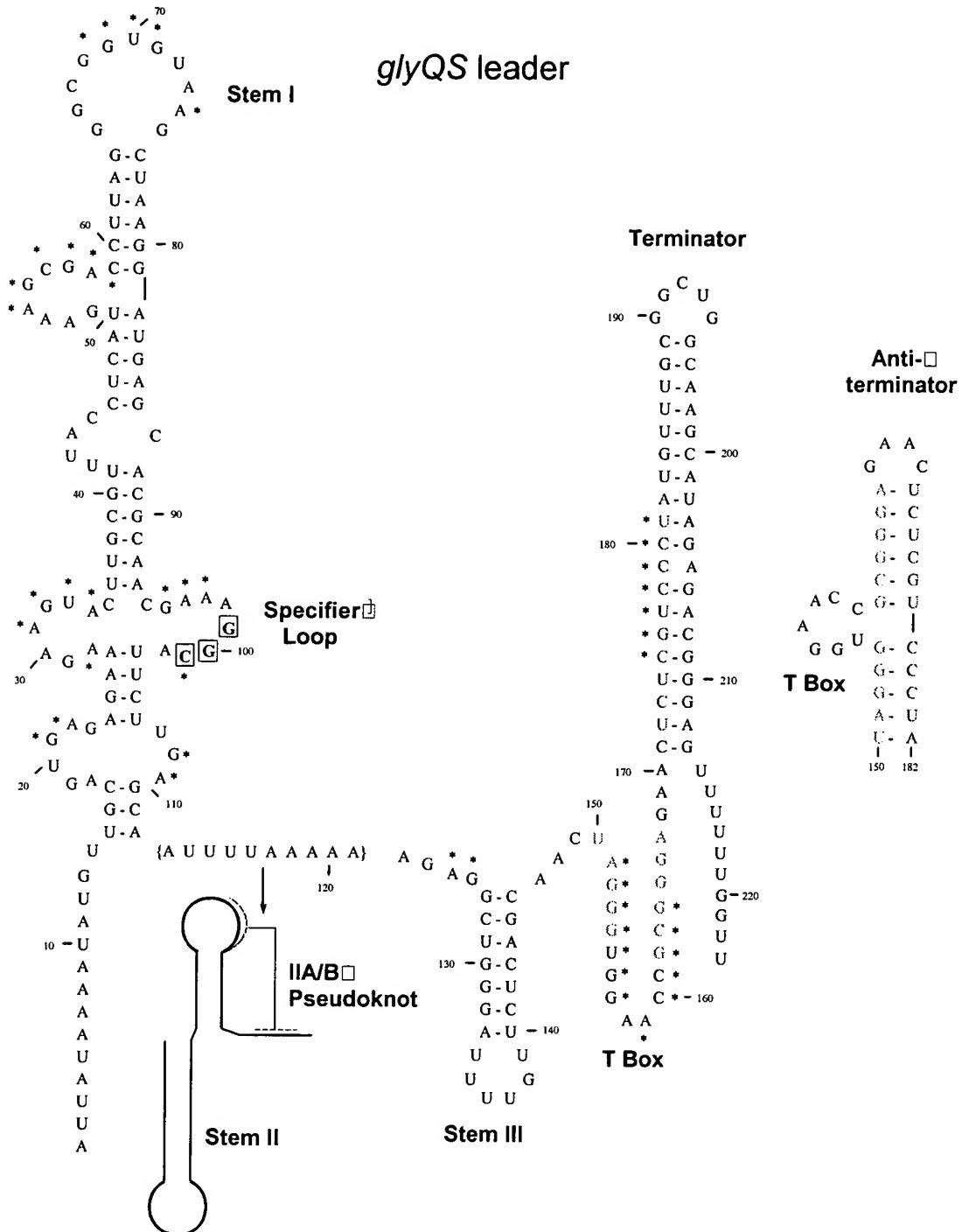


FIG. 2

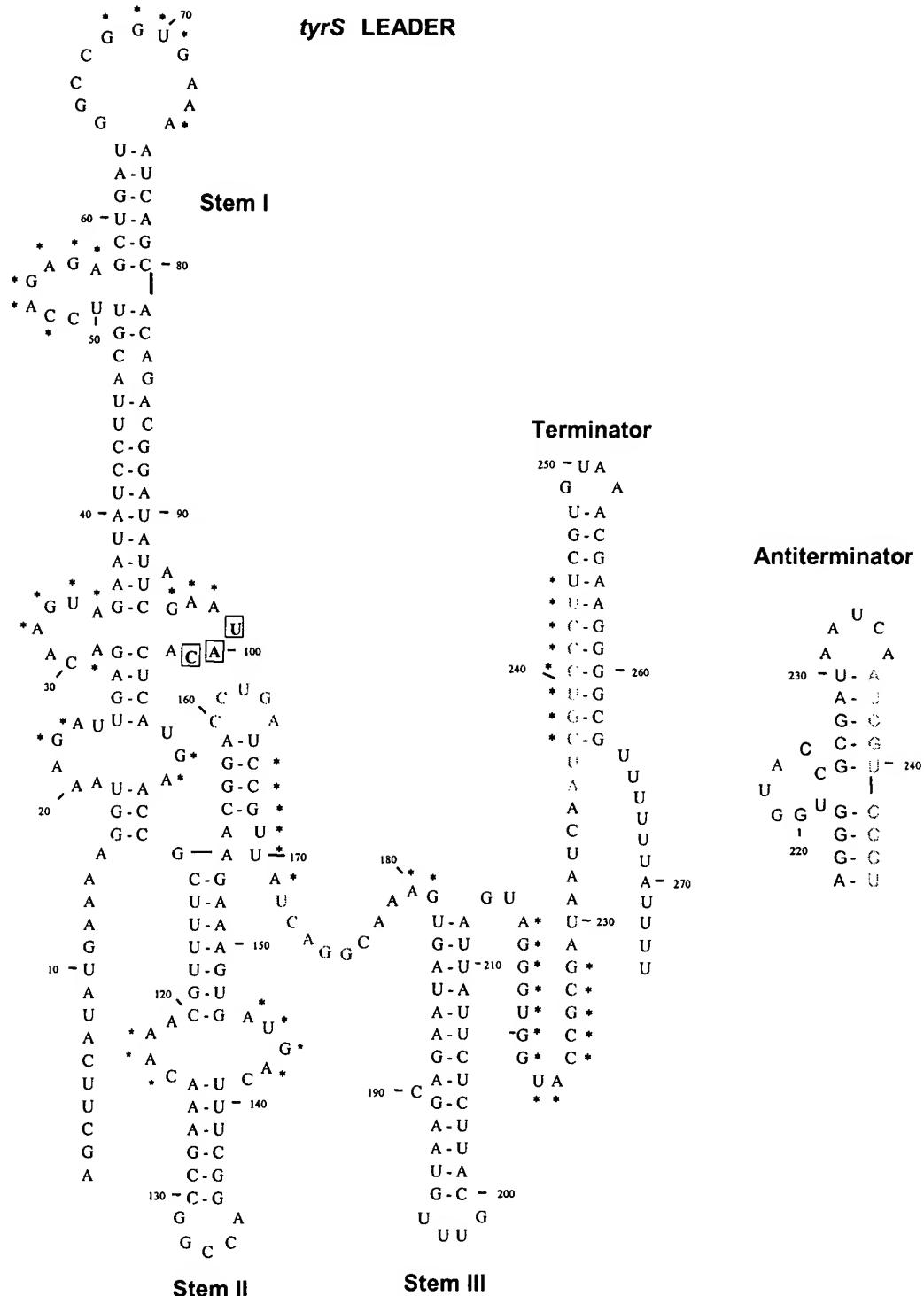


FIG. 3: Panel A

B. sub	<u>TyrS</u>	AGTTTCATATGAAAGGTAAGAT TCAGA - CAAGTAGA - ATATCCCT -	TACGT - TCCAGAGA - GCT - GAT
B. ant	<u>glys</u>	ATTATTAAATAATAGTACGGATGACG TA - TATAAGTACTTGC -	<u>ACA</u> - AAAAGCGA - TTC - AGG
B. cer	<u>glys</u>	ATTATTAAATAATAGTACGCTATGACG GA - TATAAGTACTTGC -	<u>ACA</u> - AAAAGCGA - TTC - AGG
B. hal	<u>glys</u>	AATGTTTAAATTCATGCTATGACG GA - ACGATCTGATTCCT -	TTCAT - AAAAGCGA - ACC - TAG
B. ste	<u>glys</u>	AATATCATATATGGATCCGGATGACG ATC - AATAGTACTTAACTCT -	TCTTC - CCAAGCGA - GCC - GGG
B. sub	<u>glys</u>	TATTTAAATATGTTGAGTGA GAA - GAAGTACTTGCCTTA -	CCCAT - GAAAGCGA - CCT - TAG
C. ace	<u>glys</u>	ATAATTAAATATCTTACAATGAC AA - AGAAATTTGATTTT -	CTT - CAAAGAGA - GGC - TGT
C. hyd	<u>glys</u>	AATTAATTAATGGATTGGCAGTGA ACCGA - GGAGTACTTGTGATT -	CCCT - TAAAGAGA - GGC - GGG
D. rad	<u>glys</u>	- GGAGGGCGTTGA ACCGA - GGAGTACCG - CCAAGA -	<u>GCCCCA</u> - ACGAGCGA - GCC - TGA
E. fae	<u>glys</u>	GAGAAGTAAATACGTACGAA AA - GAAGTAAAGAA AA -	<u>GCCCCC</u> - TCTGTT - AAGCGA - TCT - GG
I. lac	<u>glys</u>	GCCITGACAAAATGGAAAATAGGCTAG - AATTTATA -	GGT - AGCGA - CTC - GAA
I. mon	<u>glys</u>	ATATCAAATTAATAGGTACGGTTGA GAA - ATAGTAACAA AA -	<u>CT</u> - AT - TTTTAGCGA - GTC - CGG
S. aur	<u>glys</u>	ATGTCACAAACATTAATTTCATTC GCC - TTTAAATAACTATCAA -	<u>TT</u> - GTACAGCGA - GTC - AAG
S. equ	<u>glys</u>	TTTGTGATAAACTAACCAAA AA - AAATAGCAGG -	TTTC T GTATC - TAAAGCGA - GCT - CGG
S. mut	<u>glys</u>	- GTTA AA AGA - GAGT - TTGT -	GGCGTTTC - TGAGCGA - ACC - TGA
S. pne	<u>glys</u>	ATTTTTGATAATAGTCAGCAG AA - AGTCTT - AT -	GGCGTTCTP - TCAAGCGA - GCT - TGG
S. pyo	<u>glys</u>	GCTTATGGCTAACTAGACTCTAGAAA - AGGATGCAAG -	TATCTTATC - TAAAGCGA - GTC - CGG

FIG. 3: Panel B

B. sub	<u>TyrS</u>	--- GGCGGGTGAAA - ATC - AGCACAGA -	CGGATATATC - GAA - TACA - CTCATGAA CC -
B. ant	<u>glys</u>	--- GATAGTGAAG - CCT - GAAGCC -	GCAG - GAA - ACGGCA - GTC T CGACG -
B. cer	<u>glys</u>	--- GATAGTGAAG - CCT - GAAGCC -	GCAG - GAA - ACGGCA - GTC T CGACG -
B. hal	<u>glys</u>	--- GATGGGTGAGA - CTC - GGGATGTA A -	CATCAG - GAA - GGCA - CTC T GGAGCA - TGA -
B. ste	<u>glys</u>	--- GACGGGGGAAG - CCC - GGGGAGA -	TGGTTAT - GAA - ACGGCA - GTC T GGAGCG -
B. sub	<u>glys</u>	--- GGGGGGTGTAAG - CTA - AGGATGAG -	CACGAAAC - GAA - AGGCA - TTC T GGAGCA -
C. ace	<u>glys</u>	--- GGAGGGGTGTA - ACG - GTCAAC -	AAAATTCTGCT - AGT - GGAG - TCTTC T GGAGTA -
C. hyd	<u>glys</u>	--- GGCTGGGTGTGA - ACC - GGTAGG -	A - TAAACGT - GAA - GGCG - CTC T GGAGCG -
D. rad	<u>glys</u>	--- GACGGGTGAGAG - TCA - GGAGGGGTGA -	GGCGGGAGGG - GAA - AGGCA - GTC T GGAGCG -
E. fae	<u>glys</u>	--- GAGAGTGGGAG - CC - AGAAACA -	CGGAACTTTT - GAA - AGGCA - CTC T GGAGTAGC -
L. lac	<u>glys</u>	--- GATAGTGAAAG - TTC - GAGAAC -	ATAAA - T - GGCT - TACTT -
L. mon	<u>glys</u>	--- GTTGGGTGTGAG - CCG - GATATTA -	ACTTTTGT - GAA - GGCG - TCTGGAGTA -
S. aur	<u>glys</u>	--- GATAGTGTAAG - CTT - AACAA -	T - AAGATT - GGC - GCGAACGATC -
S. equ	<u>glys</u>	--- GGCTGGGTGAGAG - CCG - AGTGTAT GG - T -	GGCG - CTC T CTA -
S. mut	<u>glys</u>	--- GAGAGTGTAAAG - TCA - GGTGAACAA -	ATAAAAGGACT - GGC A - CTC T CTA -
S. pne	<u>glys</u>	--- GATAGTGGGAG - CCA - AGTAGGGCAA -	ATAAAAGGCT - GGCG - CTC T CTA -
S. pyo	<u>glys</u>	--- GGTAGTTAGAG - CCG - AA TGG TAGGA -	CTGCAAGATT - GGCG - CTC T CTA -

FIG. 3: Panel C

B. sub	tyrS	GCT	-TTTGC-	-AAAC-	-AAGCC-	GGCCA-	-GGCTTT-	-CAGTA-	- <u>GTGAA</u> -	-AGA
B. ant	glyS	TTT	-	-	-	-ATACTGATAA-	-	-	-	-
B. cer	glyS	GCA	-	-	-	-AATACGTGATAA-	-	-	-	-
B. hal	glyS	GGAGAAA	-	-	-	-AATACTGATAA-	-	-	-	-
B. ste	glyS	CGATGAAAAGAA-	-	-	-	-CGATGAAAAGAA-	-	-	-	-
B. sub	glyS	GAAATGGCAAAA-	-	-	-	-GAAATGGCAAAA-	-	-	-	-
C. ace	glyS	TTT	-	-	-	-TT-	-	-AAAA-	-GA	-AAA
C. hyd	glyS	GGAGGAAA	-	-	-	-GCAAG-	-	-GGC	-AGTAAAGGCC-	-TGC
D. rad	glyS	CCC	-	-	-	-	-	-	-	-
E. fae	glyS	-	-	-	-	-	-	-	-	-
L. lac	glyS	AAAACGTAAATGAAAC	-	-	-	-ACAAAT-	-	-AAAGTAAAAAA-	-	-
L. mon	glyS	CAGCGAAATCAA-	-	-	-	-	-	-	-	-
S. aur	glyS	ATTTAAATATAAA-	-	-	-	-	-	-	-	-
S. equ	glyS	-AAGAGTAGGCTCA-	-	-	-	-	-	-	-	-
S. mut	glyS	CTT	-	-	-	-AAT-	-	-AGGCC-	-AAG	-
S. pne	glyS	GGCT	-	-	-	-	-	-	-	-
S. pyo	glyS	TATA	-	-	-	-	-	-	-	-
		GCG	-CAGTGT-	-GATTAG-	-	-	-	-	-	-

FIG. 3: Panel D

B. sub	tyrS	-ACGGA-	-CCTGA-	-TCCGTTATCAGG-	-	-	-	-	-	-
B. ant	glyS	TTT	-	-	-	-	-	-	-	-
B. cer	glyS	-	-	-	-	-	-	-	-	-
B. hal	glyS	-	-	-	-	-	-	-	-	-
B. ste	glyS	-	-	-	-	-	-	-	-	-
B. sub	glyS	-	-	-	-	-	-	-	-	-
C. ace	glyS	-	-	-	-	-	-	-	-	-
C. hyd	glyS	-	-	-	-	-	-	-	-	-
D. rad	glyS	-	-	-	-	-	-	-	-	-
E. fae	glyS	-	-	-	-	-	-	-	-	-
L. lac	glyS	-	-	-	-	-	-	-	-	-
L. mon	glyS	-	-	-	-	-	-	-	-	-
S. aur	glyS	-	-	-	-	-	-	-	-	-
S. equ	glyS	-	-	-	-	-	-	-	-	-
S. mut	glyS	-	-	-	-	-	-	-	-	-
S. pne	glyS	-	-	-	-	-	-	-	-	-
S. pyo	glyS	-	-	-	-	-	-	-	-	-

FIG. 3: Panel E

B. sub	<u>tyrs</u>	AAAGTGA	-----	TAAGACGAATGTTG	-----	CATTCA-TCTTA	-----	TTA
B. ant	<u>glyS</u>	AGTGGAT	-----	GCACCTTT	-----	GRGT	-----	ATC
B. cer	<u>glyS</u>	AGTGGAT	-----	GCACCTTT	-----	GTGT	-----	ATC
B. hal	<u>glyS</u>	AGTGGCC	-----	TATGGTGTG	-----	ATCATA	-----	GGC
B. ste	<u>glyS</u>	AGGGGAT	-----	GGGTGATT	-----	TGCGC	-----	ATC
B. sub	<u>glyS</u>	TTTAAAAAGAGGCT	-----	GGGATTTGT	-----	TCTC	-----	AGC
C. ace	<u>glyS</u>	AGAGTTTGAGGTGGGC	-----	CTTTTTT	-----	G	-----	GCC
C. hyd	<u>glyS</u>	AGAGTTTGAGGTGGGC	-----	GCGAGG	-----	GC	-----	CAG
D. rad	<u>glyS</u>	ACAAACGGTCTGAAGGTGCTG	-----	GCAGATGAACAA	-----	CATCGG	-----	AGG
E. fae	<u>glyS</u>	ACAAACGAAAGCT	-----		-----		-----	
L. lac	<u>glyS</u>	GGTGGGA	-----	ATTGTTT	-----	TAAT	-----	TCC
L. mon	<u>glyS</u>	AGCGAGT	-----	GAATA	-----	C	-----	ACT
S. aur	<u>glyS</u>	GGTGGTT	-----	GTAGCTTGCTGACATCTGTT	-----	TATCAA-CAAAG-ATC	-----	AAA
S. equ	<u>glyS</u>	CTAACAA	-----	ATCAGATAAA	-----	TGA	-----	AGT
S. mut	<u>glyS</u>	GTAGTAT	-----	T	-----	TGAAGTA	-----	ATA
S. pne	<u>glyS</u>	AAAAAGATAAC	-----	TATATAAA	-----	TGA	-----	GTA
S. pyo	<u>glyS</u>		-----		-----		-----	

FIG. 3: Panel F

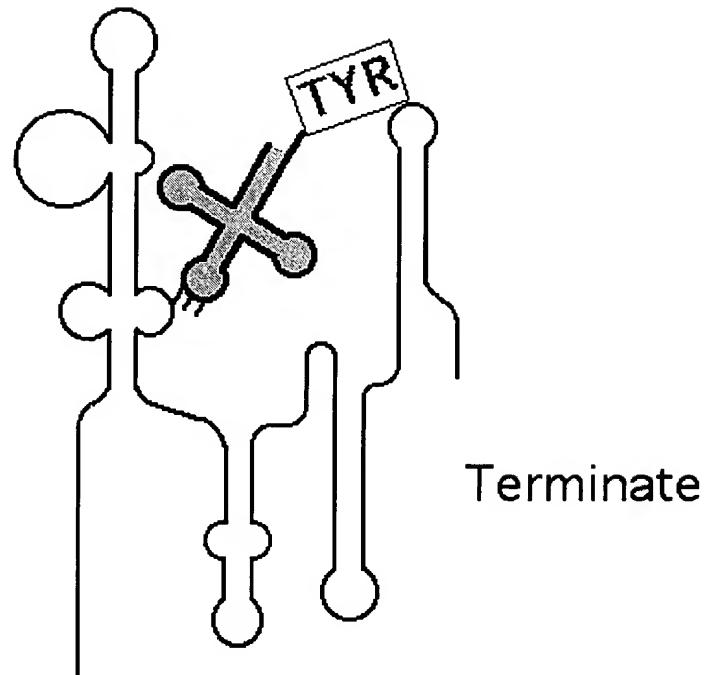
B. sub	<u>tyrs</u>	GTAGGGTGGTACCGCGAT	-----	AATCA	-----	ATCGT	-----	CCCT
B. ant	<u>glyS</u>	AACTAGGGTGGAAACCGCGGC	-----	AAAC	-----	CTCGT	-----	CCCTAG
B. cer	<u>glyS</u>	AACTAGGGTGGAAACCGCGGC	-----	AAAC	-----	CTCGT	-----	CCCTAG
B. hal	<u>glyS</u>	AACTAGGGTGGAAACCGCGGC	-----	TTAACT	-----	CTCGT	-----	CCCTAT
B. ste	<u>glyS</u>	AACTAGGGTGGAAACCGCGGC	-----	TAC	-----	GCTCTCGT	-----	CCCTAG
B. sub	<u>glyS</u>	AACTAGGGTGGAAACCGCGGC	-----	GAAC	-----	TCTCGT	-----	CCCTA
C. ace	<u>glyS</u>	AACTAGGGTGGAAACCGCGGA	-----	GTAA	-----	TCTCGT	-----	CCCTT
C. hyd	<u>glyS</u>	AACCAAGGGTGGAAACCGCGGAAGG	-----	ATGCC	-----	CCTTTCGT	-----	CCCTGG
D. rad	<u>glyS</u>	AACTGGGGTGGAAACCGCGCATG	-----	TCC	-----	CCTGGGT	-----	CCCGGG
E. fae	<u>glyS</u>	TAGGATGGAAACCGCGGA	-----	TAATTAT	-----	TCTG	-----	CCCTA
L. lac	<u>glyS</u>	ATAAAGGGTGGAAACCGCGGA	-----	ATTT	-----	GCAC	-----	CCTTGT
L. mon	<u>glyS</u>	ATAAAGGGTGGAAACCGCGGA	-----	CTAACT	-----	CTCGT	-----	CCCTAT
S. aur	<u>glyS</u>	ATTTGGGGTGGAAACCGCGGT*	-----	TTTG	-----	AACTCGT	-----	CCCTA
S. mut	<u>glyS</u>	TGAAGTAATAATAAGGGTGGAAACCGCGGT	-----		-----	ACGC	-----	CCCTA
S. pne	<u>glyS</u>	ATAAAATAGGGTGGAAACCGCGGT	-----	TCA	-----	AACGC	-----	CCCTA
S. pyo	<u>glyS</u>	ATAAAATAGGGTGGAAACCGCGGT	-----	TCT	-----	GACGC	-----	CCCTAG
		ATAAAATAGGGTGGAAACCGCGGT	-----	TTTG	-----	ACGC	-----	CCCTAG

FIG. 4

ORGANISMS WITH LEADER SEQUENCES HAVING THE T BOX PATTERN		NCBI GENOME SEQUENCE FILE NAME
5		
	<i>Bacillus anthracis</i>	NC_003997
	<i>Bacillus cereus</i>	NC_004722
	<i>Bacillus halodurans</i>	NC_002570
	<i>Bacillus subtilis</i>	NC_000964
10	<i>Carboxydothermus hydrogenoformans</i>	NC_002972
	<i>Clostridium acetobutylicum</i>	NC_003030
	<i>Clostridium botulinum</i>	NC_003223
	<i>Clostridium difficile</i>	NC_002933
	<i>Clostridium perfringens</i>	NC_003366
15	<i>Clostridium tetani</i>	NC_004557
	<i>Clostridium thermocellum</i>	NZ_AABG00000000
	<i>Corynebacterium glutamicum</i>	NC_003450
	<i>Corynebacterium diphtheriae</i>	NC_002935
	<i>Deinococcus radiodurans</i>	NC_001263
20	<i>Desulfitobacterium hafniense</i>	NZ_AAAW00000000
	<i>Enterococcus faecalis</i>	NC_004668
	<i>Enterococcus faecium</i>	NZ_AAAK00000000
	<i>Geobacillus stearothermophilus</i>	NC_002926
	<i>Geobacter sulfurreducens</i>	NC_002939
25	<i>Lactococcus lactis</i>	NC_002662
	<i>Leuconostoc mesenteroides</i>	NZ_AABH00000000
	<i>Listeria innocua</i>	NC_003212
	<i>Listeria monocytogenes</i>	NC_003210
	<i>Mycobacterium leprae</i>	NC_002677
30	<i>Mycobacterium tuberculosis</i>	NC_000962
	<i>Oceanobacillus iheyensis</i>	NC_004193
	<i>Oenococcus oeni</i>	NZ_AAAAZ00000000
	<i>Ruminococcus albus</i>	NC_003373
	<i>Spiroplasma kunkelii</i>	NC_003999
35	<i>Staphylococcus aureus</i>	NC_002758
	<i>Staphylococcus epidermidis</i>	NC_004461
	<i>Streptococcus equi</i>	NC_002955
	<i>Streptococcus gordoniae</i>	NC_002979
	<i>Streptococcus mutans</i>	NC_004350
40	<i>Streptococcus pneumoniae</i>	NC_003098
	<i>Streptococcus pyogenes</i>	NC_002737
	<i>Streptomyces coelicolor</i>	NC_003888
	<i>Thermoanaerobacter tengcongensis</i>	NC_003869
	<i>Thermobifida fusca</i>	NZ_AAAQ00000000

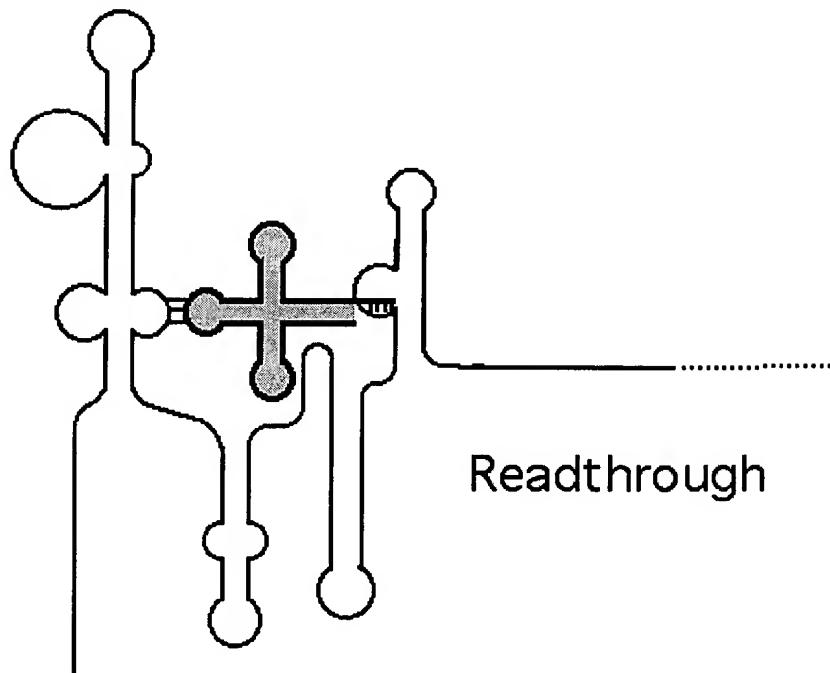
FIG. 5

High tRNA
charging



Terminate

Low tRNA
charging



Readthrough

FIG. 6

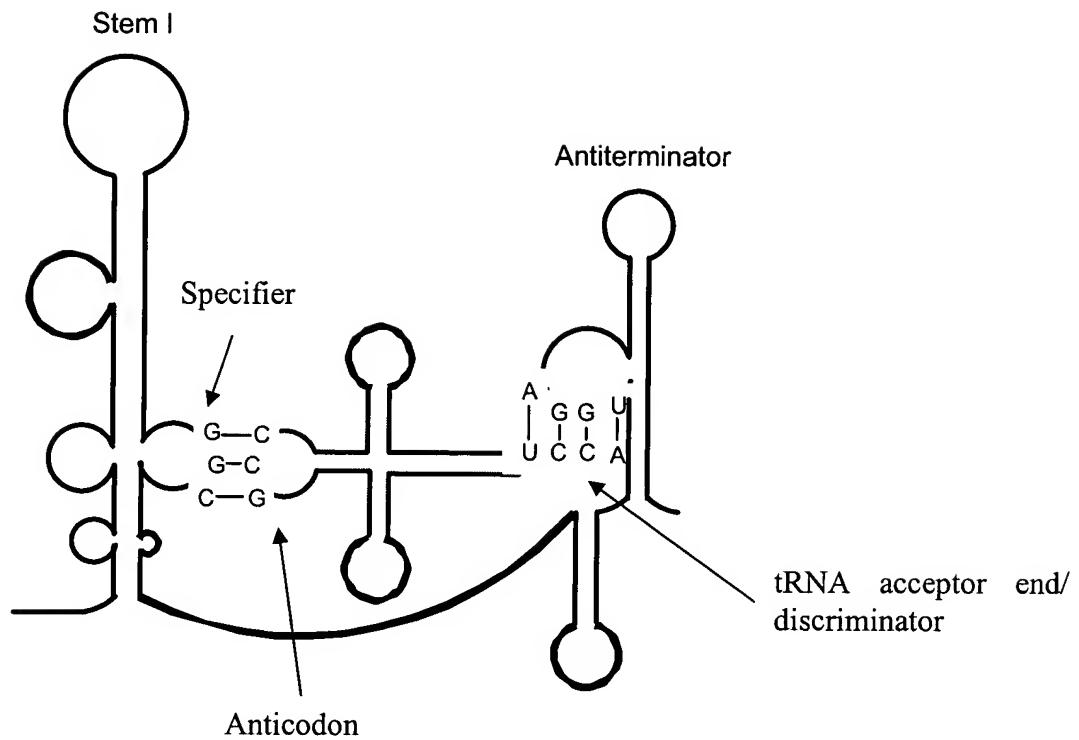


FIG. 7

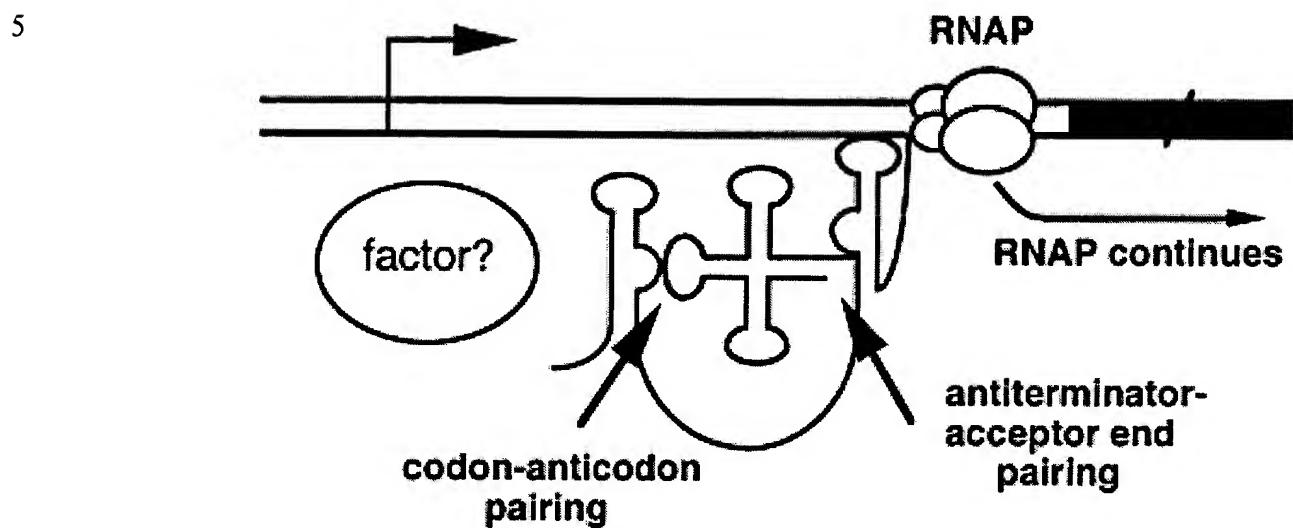


FIG. 8

Diagram illustrating a bacterial promoter region with transcription start sites and RNA lengths. The sequence is: TTGACATTTGGTCCATCTTTTATATGATCATTATTATAAAAATATGTTGC. Key features include:

- Promoter Elements:** -35 and -10 boxes are indicated above the sequence.
- Start Site:** The +1 start site is marked with a 'T' below the sequence.
- Transcripts:**
 - A top line represents a transcript of 215 nt, starting from the +1 site.
 - A bottom line represents a transcript of 305 nt, starting from the +1 site.

FIG. 9

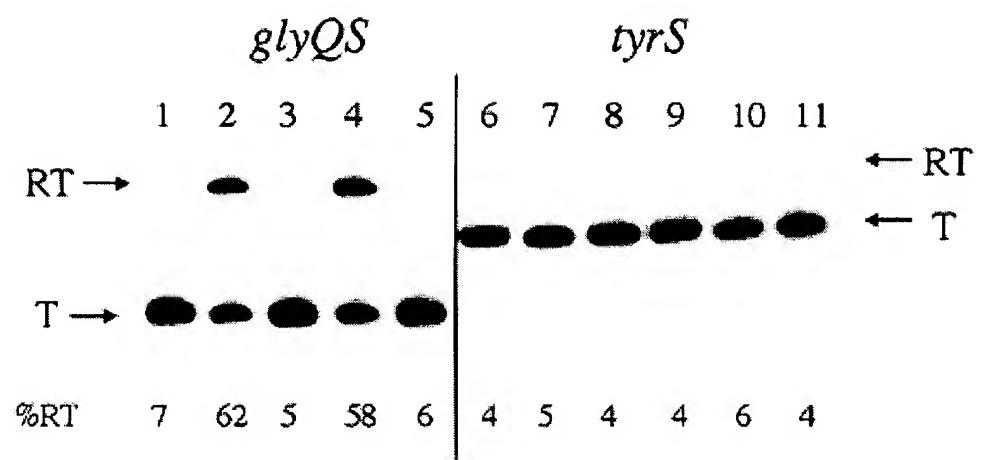


FIG. 10

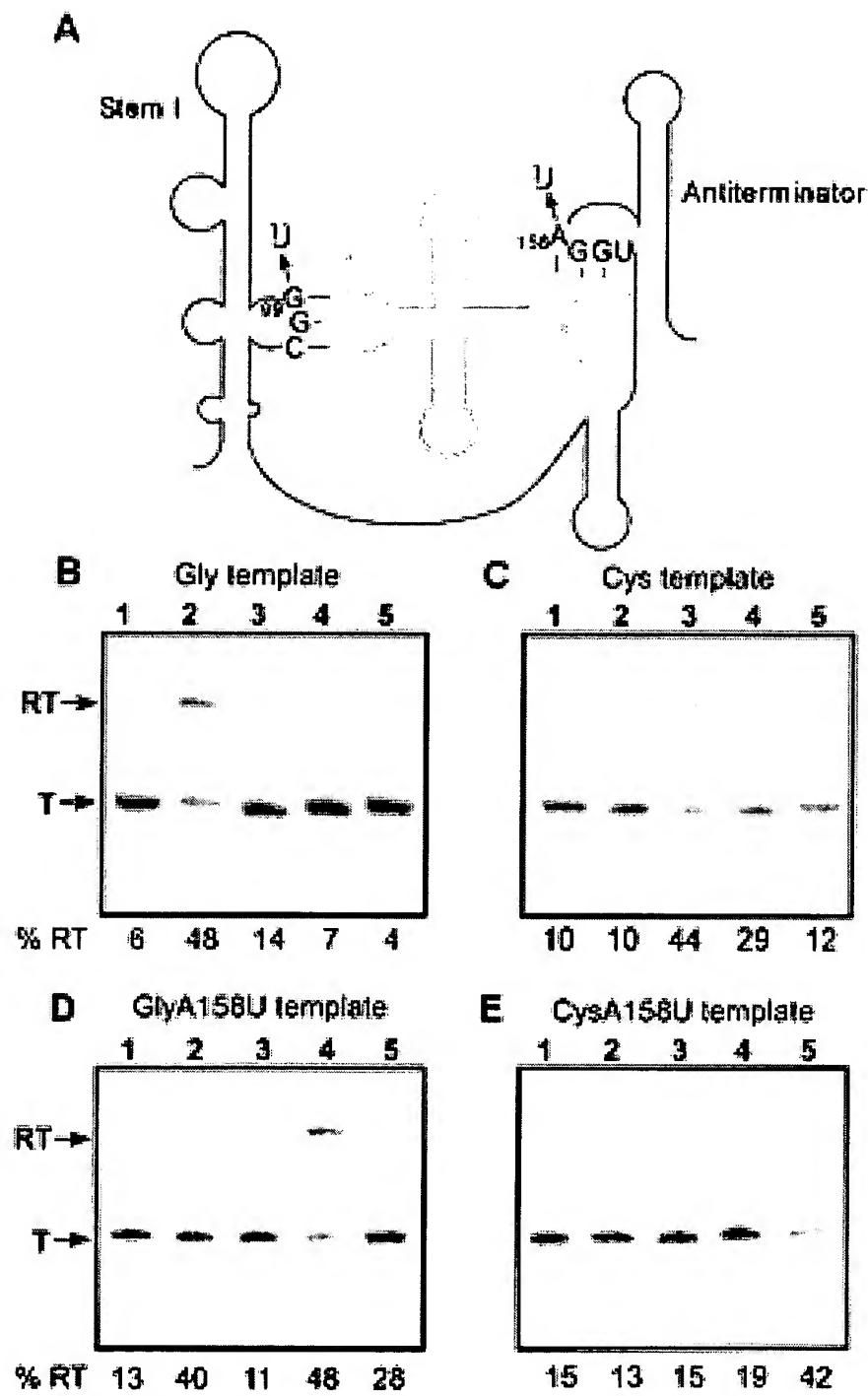


FIG. 11

Polynucleotide sequence for the *glyQS* gene from *Bacillus subtilis* corresponding to the *in vitro* transcription template: from *B. subtilis* 168.

```
ATTGATTTATTAACGAAGAATATCGGGATTGTATTAAAATCAAAGCGTTTTAGATCAAATGGAAAGCATGAA
ACATCTTATGGGTGAAAACAAAAGTTGACATTGGTCATCTTTTATATGATCATTATTAAATATGTTGCAG
TGAGAGAAAGAAGTACTTGCCTTACCTCATGAAAGCGACCTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGA
AAGGCATTCTTGAGCAATTTAAAAAGAGGCTGGGATTTGTTCTCAGCAACTAGGGTGGAACCGCGGGAGAACTC
TCGTCCCTATGTTGCGGCTGGCAAGCATAAGAGACGGGAGTTTTGGTTGCTGCCGCAGTCAACTTATGAAAGAAA
AGTGGAGGTGCTTGAAATGAATATTCAAGACATGATTCTAACCTTGCAGAAAGC
```

FIG. 12

Sequence of same region from BR151MA (T to A at +6 position relative to transcription start-site)

```
ATTGATTTATATTACGAAGAATATTGGGATTGTATTTAAAATCAAAGCGTTTTAGATCAAATGGAAAGCATGAA
ACATCTTATGGGTGAAAACAAAAGTTGACATTGGTCATCTTTTATATGATCATTATTATAAAAATATGTTGCAG
TGAGAGAAAGAAGTACTTGCCTTACCTCATGAAAGCGACCTTAGGGCGGTGAAGCTAAGGATGAGCACGCAACGA
AAGGCATTCTTGAGCAATTAAAAAGAGGCTGGGATTTGTCTCAGCAACTAGGGTGGAACCGCGGGAGAACTC
TCGTCCTATGTTGCGGCTGGCAAGCATAAGACGGGAGTTTTGGTTGCTGCCAGTCACATTATGAAAGAAA
AGTGGAGGTGCTTGAATGAATATTCAAGACATGATTCTAACCTGCAAAGC
```

FIG. 13

tRNA^{Gly} DNA sequence: (from SubtiList, confirmed by sequencing of region in BR151MA)

GCGGAAGTAGTCAGTGGTAGAACACCACCTGCCAAGGTGGGGTGCAGGGTTCGAATCCGTCTCCGCTCCA

FIG. 14

PCR primers used for preparing *glyQS* template:

GlyQUS1Xba: ATTGATCTAGATTACGAAGAATATCGGGATTGTA (contains two changes from sequence shown in Fig. 12 to introduce an XbaI site (TCTAGA) at the 5' end of the fragment for generation of the *glyQS-lacZ* fusion construct)

GlyQDS2H3Pac: GGGTATTTAATTAAAGCTTTGCAAGGTTAGAATCA (introduces extra 14 nt downstream of *glyQS* sequence shown in Fig. 12 to provide a HindIII site (AAGCTT) for generation of the *glyQS-lacZ* fusion construct)

The PCR primers used to generate the *tyrS* template:

TyrUSBM: GGCTGGGGATCCGTCAACAATGGAGG

TyrN2b: CCGCGGAAGGATAAAGCTTCAAGTAAG

FIG. 15

Polynucleotide sequence for the *tyrS* template DNA (identical for 168 or BR151MA).

GGCTGGAGATCTGTCAACAATGGAGGATTAAGGCGCGTTGACACAGGTTTATTATGTTAAAATGATATAG
CTTCATATGAAAAGTAAAGATTGAGACAAGTAGAATATCCTTACGTTCCAGAGAGCTGATGGCCGGTGAAAATCAG
CACAGACGGATATATCGAATACACTCATGAACCGCTTTGCAAACAAAGCCGCCAGGCTTCAGTAGTGAAAGAAC
GGACCTGATCCGTTATCAGGCAAAGTGATAAGACGAATGTTGCATTCTTATTAGTAGGGTGGTACCGCGATAAT
CAATCGTCCCTCGTGTAAACGAAGGGCGTTTTTATTAAATTAAAAAGGAGCTTATCTTATGACTAACTTAC
TTGAAGACTTATCCTCCGCGG

FIG. 16

The oligos used to generate tRNA^{Tyr}:

5': TyrUSBM: GGCTGGGATCCGTCAACAATGGAGG
3': TyrN2b: CCGCGGAAGGATAAAGCTTCAAGTAAG

5': tRNATyrUST7: TAATACGACTCACTATAGGAGGGTAGCG (the tRNA sequence starts with GGAGGGTAGCG)
3': tRNATyrDS: TGGTGGAGGGGGCAGATTCCG

The oligos used to generate tRNA^{Gly}:

5': GlytRNAUS: TAATACGACTCACTATAGCGGAAGTAGTCAGTGG
(the first part of this oligo is the T7 promoter; the tRNA starts with GCGGAAGTAGTCAGTGG)
3': GlytRNADS: TGGAGCGGAAGACGGGATTCGAAC

FIG. 17

tRNA^{Tyr} DNA sequence.

GGAGGGGTAGCGAAGTGGCTAACGCGCGGACTGTAAATCCGCTCCCTCAGGGTTCGGCAGTCGAATCTGCC
CTCCACCA